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First Genome-Wide Association Study of Latent Autoimmune Diabetes in Adults Reveals Novel Insights Linking Immune and Metabolic Diabetes

Bone Mineral Density Childhood

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OBJECTIVE

Latent autoimmune diabetes in adults (LADA) shares clinical features with both type 1 and type 2 diabetes; however, there is ongoing debate regarding the precise definition of LADA. Understanding its genetic basis is one potential strategy to gain insight into appropriate classification of this diabetes subtype.

RESEARCH DESIGN AND METHODS

We performed the first genome-wide association study of LADA in case subjects of European ancestry versus population control subjects ($n = 2,634$ vs. $5,947$) and compared against both case subjects with type 1 diabetes ($n = 2,454$ vs. 968) and type 2 diabetes ($n = 2,779$ vs. $10,396$).

RESULTS

The leading genetic signals were principally shared with type 1 diabetes, although we observed positive genetic correlations genome-wide with both type 1 and type 2 diabetes. Additionally, we observed a novel independent signal at the known type 1 diabetes locus harboring *PFKFB3*, encoding a regulator of glycolysis and insulin signaling in type 2 diabetes and inflammation and autophagy in autoimmune disease, as well as an attenuation of key type 1–associated HLA haplotype frequencies in LADA, suggesting that these are factors that distinguish childhood-onset type 1 diabetes from adult autoimmune diabetes.

CONCLUSIONS

Our results support the need for further investigations of the genetic factors that distinguish forms of autoimmune diabetes as well as more precise classification strategies.

The relationship between latent autoimmune diabetes in adults (LADA) and both type 1 and type 2 diabetes is not fully elucidated and not appropriately encapsulated in the term “type 1.5 diabetes” (1–3). In many populations, LADA is at least as prevalent as childhood-onset type 1 diabetes (4) but is frequently misdiagnosed as type 2 diabetes (5,6) given its presentation in adults without need for insulin. As such, subjects with LADA could be present in cohort studies for type 2 diabetes that do not screen out autoantibody-positive case subjects, potentially resulting in the identification of genetic associations for type 2 diabetes that are etiologically related to

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autoimmunity. Furthermore, LADA has a natural history distinct from that of type 2 diabetes and is likely mismanaged as a result (5). The challenge to define adult autoimmune diabetes, including LADA, as distinct from the generality of type 2 diabetes is acute given the increasingly larger data sets assembled to identify additional, common genetic risk factors of increasingly smaller effect sizes. Indeed, reflecting this concern, recent genome-wide association study (GWAS) analyses of type 2 diabetes have reported associations at type 1 diabetes-associated regions such as *HLA-DQA1* in populations of European ancestry (7) and *HLA-B* and *INS-IGF2* in populations of African ancestry (8). As such, understanding the genetic etiology of adult autoimmune diabetes will not only aid the characterization of this relatively common form of diabetes, but will also facilitate our understanding of both type 1 and type 2 diabetes.

To date, the relatively limited candidate gene studies carried out for LADA have supported a role for both type 1 and type 2 diabetes risk loci (1,9–15). Most notable from these previous studies is the implicated role of the key type 2 diabetes-associated *TCF7L2* locus in the pathogenesis of LADA (11,13,16). More

recently, we constructed genetic risk scores combining known type 1 and type 2 diabetes loci and assessed their impact in LADA, and our results implicated a role for both sets of loci (12). However, no systematic genome-wide appraisal of adult autoimmune diabetes has been performed. Therefore, in this study, we performed the first GWAS of LADA against population control subjects and further contrasted LADA against type 1 and type 2 diabetes to better understand its genomic signature in comparison with these two better characterized forms of diabetes.

RESEARCH DESIGN AND METHODS

Study Subjects

Case subjects diagnosed with LADA were included from cohorts of European ancestry (Supplementary Table 1), including Action LADA (includes samples from the U.K., Germany, and U.S.), All New Diabetics In Scania (ANDIS), the Botnia Study, Copenhagen LADA (includes samples from the Danish Centre for Strategic Research in Type 2 Diabetes, Vejle Diabetes Biobank, Odense University Hospital, Copenhagen Insulin and Metformin Therapy Trial, Inter99, Steno Diabetes Center), the Diabetes Registry Vaasa (DIREVA), Genetics of Diabetes Audit and Research

in Tayside Scotland (GoDARTS), Nord-Trøndelag Health Study (HUNT), and Scania Diabetes Registry (SDR). Control subjects were population-based (including samples from the Bone Mineral Density in Childhood Study [BMDCS], Copenhagen control subjects [with samples from the 1936 Birth Cohort and ADDITION-PRO], GoDARTS, HUNT, the Malmö Diet and Cancer Study, DIREVA, and SDR).

Inclusion and exclusion criteria for LADA, type 1 diabetes, type 2 diabetes, and population control subjects varied by cohort (see Supplementary Table 1 and Supplementary Data for details). In general, LADA was defined by an age at diagnosis older than 20, 30, or 35 years, with some cohorts restricting the upper age limit to 70 years; the presence of diabetes-associated autoimmune autoantibodies, in particular GAD autoantibody (GADA) positivity; and the lack of insulin requirement for 6 months or 1 year after diagnosis. In some case subjects, C-peptide level was also used as a filter. This study was approved by local institutional ethical review boards for all participating centers.

Genotyping and Imputation

Each respective cohort performed genome-wide genotyping on the Illumina CoreExome

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chip, the Illumina OmniExpressExome BeadChip, or the Affymetrix 6 array. Case and control subjects from each study center were matched on the same genotyping chip to reduce batch effects. Standard post-genotyping quality control was performed, including sample exclusions for ambiguous gender, call rate <95%, and any duplicate or related individuals ($\pi_{\text{hat}} \geq 0.2$), and single nucleotide polymorphism (SNP) exclusions for monomorphic SNPs, SNPs with minor allele frequency <0.05, and SNPs with missingness rate >0.05. The Haplotype Reference Consortium imputation service (Michigan imputation server, <https://imputationserver.sph.umich.edu/index.html>) was used to perform imputation for autosomal SNPs.

Genome-Wide Association and Meta-analysis: LADA Versus Control Subjects, LADA Versus Type 1 Diabetes, and LADA Versus Type 2 Diabetes

SNPTEST (17) or Efficient and Parallelizable Association Container Toolbox (<http://genome.sph.umich.edu/wiki/EPACTS>) was used by each respective cohort to perform case-control GWAS of LADA ($n = 2,634$) versus population control subjects ($n = 5,947$), LADA ($n = 2,454$) versus case subjects with type 1 diabetes ($n = 968$), and LADA ($n = 2,779$) versus case subjects with type 2 diabetes ($n = 10,396$), including sex and principal components as covariates (see Supplementary Table 1 for cohort-specific covariates).

After GWAS, filtering was performed centrally to include only SNPs with a minor allele frequency >0.05, INFO quality score >0.4, and a Hardy-Weinberg equilibrium $P > 1 \times 10^{-7}$. Meta-analysis was then performed for LADA versus population control subjects, LADA versus type 1 diabetes, and LADA versus type 2 diabetes with GWAMA (18) with two rounds of genomic control (Supplementary Table 2 and Supplementary Figs. 1 and 2).

Signals in the secondary tier ($P = 1 \times 10^{-6}$ to 5×10^{-8}) for the LADA versus population control subject analysis were followed up in the GoDARTS and HUNT cohorts (LADA, $n = 345$; control subjects, $n = 1,664$) and meta-analyzed with the discovery set (total LADA, $n = 2,979$; control subjects, $n = 7,611$) to assess whether any novel signals would reach genome-wide significance.

Enrichment of Directional Consistency Among Type 1 Diabetes/Type 2 Diabetes Loci in LADA

To estimate whether the concordance in direction of effects for type 1 and type 2 diabetes loci in LADA is significantly different from chance, a binomial test was used, assuming a null hypothesis of 50% agreement.

Conditional Analysis

Approximate conditional analysis for known type 1 diabetes-associated loci was carried out for the LADA versus control subject summary statistics results for the 10p15.1 locus using genome-wide complex trait analysis (19). For this locus, LADA versus control subjects plus HUNT summary statistics were conditioned on the following type 1 diabetes-associated SNPs: rs61839660 (20), rs10795791 (20), rs7090530 (21), rs12251307 (22), rs41295121 (20), and rs11258747 (22). We did not condition on the significant signals at other loci. For 12q24.3, two of the type 1 diabetes-associated SNPs (rs3184504 [22] and rs653178 [20]) were in high linkage disequilibrium ($r^2 > 0.9$) with our lead SNP. Additionally, the MHC, *PTPN22*, and *INS* loci were not conditioned, as the top signals were identified as type 1 diabetes-associated SNPs.

Stratification Analysis by GADA Titer

Case subjects with LADA are heterogeneous in terms of GADA titer (23). Therefore, to further understand the genetic landscape of LADA in the context of different GADA levels, we stratified case subjects into tertiles in Action LADA, ANDIS, DIREVA, and SDR. We performed three GWAS on 1) the top tertile with the highest GADA titers ($n = 627$) versus population control subjects ($n = 4,314$), 2) the top two tertiles with the highest GADA titers ($n = 1,012$) versus population control subjects ($n = 4,314$), and 3) the bottom tertile with the lowest GADA titers ($n = 562$) versus population control subjects ($n = 4,314$).

LD Score Regression

To test for genetic correlations genome-wide among LADA, type 1 diabetes (21), and type 2 diabetes (24), we used LD score regression through the LDSC v.1.0.0 python package (25).

Pathway Analysis

DEPICT pathway analysis (26) was used to perform gene set enrichment, tissue enrichment, and gene prioritization analyses.

HLA Imputation/Analysis

The HLA imputation software SNP2HLA (27) was used to impute chromosome 6 in Action LADA ($n = 1,365$), Swedish case subjects with LADA ($n = 794$), BMDCS ($n = 1,056$), and case subjects with type 1 diabetes from the Wellcome Trust Case Control Consortium ($n = 1,990$). HLA alleles with four-digit resolution were imputed. The R package BIGDAGW (<https://cran.r-project.org/web/packages/BIGDAGW/>) (28) was used to test for allele frequency differences for established type 1 diabetes-associated HLA haplotypes between LADA versus type 1 diabetes as well as LADA versus BMDCS. Haplotypes with frequencies <1% across LADA, type 1 diabetes, and BMDCS were removed from the analysis given that rare haplotypes can result in unstable variance estimates and unreliable test statistics.

RESULTS

Genome-Wide Association of LADA Versus Population Control Subjects

We first conducted GWAS in patients with LADA ($n = 2,634$) versus population-based control subjects ($n = 5,947$) of European ancestry in a discovery meta-analysis setting (Supplementary Table 1) (power calculations can be found in Supplementary Table 3). Four signals achieved genome-wide significance ($P < 5 \times 10^{-8}$), all at established type 1 diabetes risk loci (*HLA*, *PTPN22*, *INS*, and *SH2B3*) (Table 1 and Supplementary Figs. 1 and 2). Pathway analysis with DEPICT (26) for signals at $P < 10^{-5}$ supported a strong immune role in the pathogenesis of LADA (Supplementary Tables 4 and 5), with gene set enrichment analysis implicating abnormal cytotoxic T-cell physiology (nominal $P = 6.39 \times 10^{-7}$) as well as the mTOR subnetwork ($P = 6.03 \times 10^{-5}$) and cell cycle ($P = 1.67 \times 10^{-5}$), as also seen in a previous epigenome-wide association study of type 1 diabetes (7), and immune system tissue types, including natural killer cells and T lymphocytes (nominal $P = 0.0079$ and 0.0082 , respectively). This is consistent with previous reports of these cell types playing a role in the pathogenesis of type 1 diabetes and LADA (29,30).

Replication Supports a Novel Locus at 6-Phosphofructo-2-Kinase/ Fructose-2,6-Biphosphatase 3

Using case subjects with LADA and population samples from an additional two

Table 1—Genome-wide significant signals associated with LADA

SNP	Chromosome	Position (b37)	Reference/ other allele	Effect allele frequency (case/control subjects)	OR	95% CI	P	Gene
LADA (<i>n</i> = 2,634) vs. population control subjects (<i>n</i> = 5,947)								
rs9273368	6	32626475	A/G	0.50/0.28	3.115	2.855–3.398	7.87×10^{-143}	<i>HLA-DQB1</i>
rs2476601	1	114377568	A/G	0.159/0.102	1.717	1.539–1.915	7.21×10^{-22}	<i>PTPN22</i>
rs689	11	2182224	T/A	0.802/0.726	1.483	1.363–1.613	1.07×10^{-19}	<i>INS</i>
rs7310615	12	111865049	C/G	0.553/0.492	1.284	1.193–1.383	4.92×10^{-11}	<i>SH2B3</i>
LADA (<i>n</i> = 2,779) vs. case subjects with type 2 diabetes (<i>n</i> = 10,396)								
rs9273368	6	32626475	A/G	0.43/0.301	2.439	2.222–2.676	3.17×10^{-78}	<i>HLA-DQB1</i>
rs689	11	2182224	T/A	0.783/0.715	1.473	1.352–1.605	9.86×10^{-19}	<i>INS</i>
rs2476601	1	114377568	A/G	0.173/0.140	1.529	1.38–1.693	4.52×10^{-16}	<i>PTPN22</i>
rs3184504	12	111884608	C/T	0.544/0.52	1.24	1.151–1.336	1.77×10^{-8}	<i>SH2B3</i>
LADA (<i>n</i> = 2,454) vs. case subjects with type 1 diabetes (<i>n</i> = 968)								
rs9273368	6	32626475	A/G	0.415/0.65	0.335	0.256–0.385	8.46×10^{-40}	<i>HLA-DQB1</i>

We performed three genome-wide association approaches, first for LADA vs. population control subjects (top), then for LADA vs. type 2 diabetes (middle), and finally for LADA versus type 1 diabetes (bottom). ORs are given for the LADA risk allele, except for rs9273368 in LADA vs. type 1 diabetes, to illustrate that the type 1 diabetes risk allele was depleted in LADA.

study centers, we attempted validation of 13 signals with suggestive association ($P < 5 \times 10^{-5}$) (Supplementary Table 6). We observed a novel signal at 10p15.1 between the two established type 1 diabetes loci at *IL2RA* and *PRKCQ*, which achieved genome-wide significance (rs1983890-C, odds ratio [OR] [95% CI] = 1.16 [1.14–1.32]; $P = 3.02 \times 10^{-8}$) (Fig. 1A and B). Given that the LADA signal is situated in close proximity to known type 1 diabetes risk loci and was in moderate-to-low LD with established type 1 diabetes-associated alleles (Supplementary Table 7), we conditioned on the type 1 diabetes SNPs and observed that rs1983890 remained strongly associated with LADA (OR [95% CI] = 1.15 [1.13–1.19]; $P = 4.35 \times 10^{-8}$) (Fig. 1C). This signal reached suggestive association in a study of type 1 diabetes ($P = 1.3 \times 10^{-7}$) (21) and as such may not represent a unique LADA association. DEPICT gene prioritization analysis (26) identified the gene encoding 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*PFKFB3*), the nearest gene to the LADA signal, as the most likely functional candidate (Supplementary Table 8).

Candidate Loci for Type 1 Diabetes and Type 2 Diabetes

Some of the loci that were suggestively associated with LADA in this study overlap previously documented type 1 diabetes

associations, including rs11755527 (*BACH2*) and rs941576 (*DLK1*) (20–22), and the type 2 diabetes association at rs11888640 (*THADA*). Taking a candidate gene approach, we extracted 66 established type 1 diabetes-associated loci from the LADA versus population control subject meta-analysis and found that 17 of these yielded association with LADA after multiple-test correction ($P < 7.6 \times 10^{-4}$) (Supplementary Table 9). Taking a similar approach with 65 established type 2 diabetes loci, none surpassed the significance threshold; however, at the nominal significance level ($P < 0.05$), 11 type 1 diabetes and 11 type 2 diabetes variants were associated with LADA, all having the same direction of effect as seen for type 1 diabetes and type 2 diabetes, respectively, except for the type 2 diabetes locus *CILP2* (rs10401969-T, OR [95% CI] = 0.820 [0.726–0.927]; $P = 0.0016$) (Supplementary Table 10). On the whole, both type 1 and type 2 diabetes loci had lower *P* values in LADA than expected by chance (Supplementary Fig. 3). Approximately 90.6% of type 1 diabetes loci (Supplementary Table 9) had directional consistency in LADA ($P = 4.51 \times 10^{-12}$) and 72.3% of type 2 diabetes loci (Supplementary Table 10) had directional consistency in LADA ($P = 2.10 \times 10^{-4}$). Combining type 1 and type 2 diabetes loci, 81.4% had directional consistency in LADA ($P = 1.40 \times 10^{-13}$). Therefore, we observed a significant

enrichment of established type 1 and type 2 diabetes loci having the same directional effect in LADA.

GWAS of LADA Versus Type 2 and Type 1 Diabetes

Next, we compared LADA with type 2 diabetes at the genome-wide level. Similar to the results of LADA versus population control subjects, LADA (*n* = 2,454) versus type 2 diabetes (*n* = 10,396) yielded genome-wide significance for the same four type 1 diabetes risk loci (Table 1). We then performed a GWAS of LADA (*n* = 2,454) versus type 1 diabetes (*n* = 968) to assess whether any differences could be detected. Only the HLA region was significantly different between type 1 diabetes and LADA, representing a relative depletion of the lead signal in LADA when compared with type 1 diabetes (rs9273368-A, OR [95% CI] = 0.335 [0.256–0.385]; $P = 8.46 \times 10^{-40}$) (Table 1). Leveraging the entire genome-wide summary statistics, genetic correlation analyses showed that LADA was positively correlated with both type 1 diabetes (with the inclusion of the HLA; r_g [SE] = 0.385 [0.136]; $P = 0.0047$) and type 2 diabetes (without the HLA; r_g [SE] = 0.281 [0.106]; $P = 0.008$).

Stratified GWAS of LADA by GADA Tertile

Stratifying LADA case subjects into tertiles resulted in the detection of the same four loci, although the magnitude of the

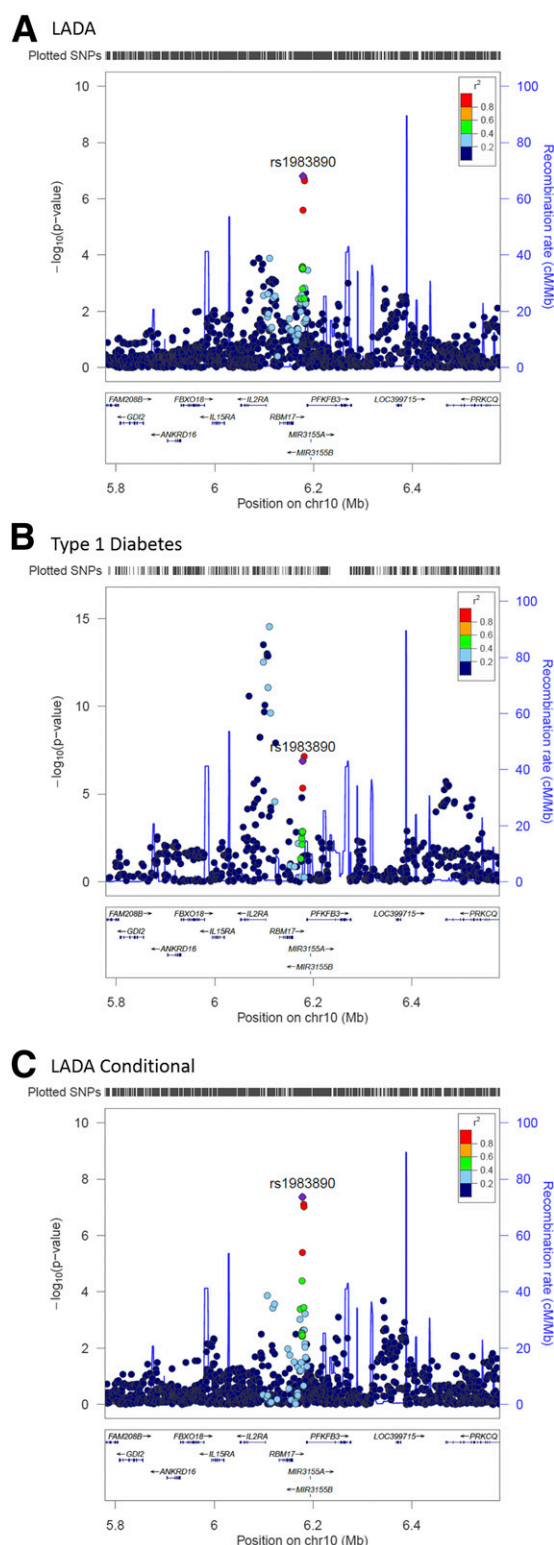


Figure 1—LocusZoom plots for the *PFKFB3* locus. **A**: In LADA vs. population control subjects with the addition of replication samples, rs1983890 reached borderline genome-wide significance. **B**: This signal lies in between two type 1 diabetes-associated loci at 10p15.1 (21). **C**: When we conditioned on the two known type 1 diabetes loci, the signal in LADA remained. LocusZoom plots were constructed to show the association data of SNPs 400 kb upstream and downstream of the lead LADA-associated signal at rs1983890. chr10, chromosome 10.

associations differed between the top tertile versus population control subjects, the top two tertiles versus population control subjects, and the bottom tertile versus population control subjects (Supplementary Table 11). As expected, the ORs for the leading loci were strongest in the case subjects with LADA with the highest GADA titers. For example, rs9273368 (*HLA-DQB1*) showed the strongest association with LADA in the analysis including the top tertile of GADA titer (OR [95% CI] = 3.30 [2.81–3.88]; $P = 1.89 \times 10^{-47}$) and the lowest association in the bottom GADA tertile (OR [95% CI] = 2.42 [2.06–2.85]; $P = 2.13 \times 10^{-26}$). Furthermore, only the *HLA-DQB1* locus was significantly associated in the case subjects with LADA with the lowest GADA titers, whereas the *PTPN22*, *INS*, and *SH2B3* loci were only evident among case subjects with higher GADA titers. Furthermore, rs7903146 at *TCF7L2* had a slightly higher OR in the group with the lowest GADA titer than that with the highest GADA titer (1.09 vs. 1.05, respectively).

HLA Haplotype Analysis

To further investigate differences in the HLA region between LADA and type 1 diabetes, we imputed this region using SNP2HLA (27) in 2,159 case subjects with LADA from the Action LADA plus Children's Hospital of Philadelphia plus Swedish cohorts and 1,990 patients with type 1 diabetes (Wellcome Trust Case Control Consortium [31]) and compared the frequencies of the leading type 1 diabetes-associated HLA haplotypes (Supplementary Table 12). After removing haplotypes with <1% frequency, 15 known type 1 diabetes-associated HLA haplotypes were tested for association in LADA compared with type 1 diabetes. Eleven type 1 diabetes haplotypes were significantly different in frequency between case subjects with LADA and type 1 diabetes after correction for multiple testing ($P < 0.003$), with all but four being protective against type 1 diabetes (32). The four type 1 diabetes susceptibility haplotypes, HLA-DRB1*0301-DQA1*0501-DQB1*0201, HLA-DRB1*0401-DQA1*0301-DQB1*0302, HLA-DRB1*0404-DQA1*0301-DQB1*0302, and HLA-DRB1*0405-DQA1*0301-DQB1*0302 (32), had significantly lower frequencies in LADA than in type 1 diabetes.

CONCLUSIONS

Taken collectively, GWAS and HLA haplotype analyses based on established associations, along with gene set enrichment analyses, support the hypothesis that the strongest genetic risk loci for LADA are shared with type 1 diabetes but that established type 2 diabetes alleles also play a weaker role, as evidenced by the enrichment of established type 2 diabetes loci in LADA and the positive genetic correlation between LADA and type 2 diabetes. The strong type 1 diabetes–like signature seen in this study in adult autoimmune diabetes could be explained by the differing genetic architectures between the two main types of diabetes (33), with type 1 diabetes having multiple low-frequency risk variants with high ORs, whereas type 2 diabetes has many common risk variants with smaller effect sizes. Given these architectural differences, any trait with a type 1 diabetes–like genetic component will detect type 1 signals first and would only subsequently detect the type 2 signals with increased statistical power (Supplementary Table 3).

Furthermore, this has important implications for genetic studies of type 2 diabetes, in which case subjects with misdiagnosed autoimmune diabetes are not routinely screened out. With increasing sample sizes and the ability to detect additional loci, type 2 diabetes GWAS that are contaminated with adult autoimmune case subjects will inevitably begin to detect type 1 diabetes–associated genetic loci, potentially misassigning these loci to type 2 diabetes etiology.

In comparing LADA to the general population, we identified a novel independent genome-wide significant signal at the *PFKFB3* locus that persisted after conditioning on the two nearby type 1 diabetes–associated signals on chromosome 10p15. Cumulative evidence for the 10p15 locus suggests it is a complex region associated with autoimmune diabetes, given that it already harbors two established risk alleles for type 1 diabetes (21,22) as well as our signal for LADA. Previous studies strongly support *PFKFB3* as a plausible biological candidate in diabetes, given its gene product's role as a regulator of glycolysis and insulin signaling (34). In mice, a pair of complementary studies showed that disrupted *PFKFB3* in adipose tissue exacerbated

insulin resistance and adipose tissue inflammation (35), whereas overexpression of the gene was protective (36). Furthermore, *PFKFB3* plays a role in autoimmune diseases; in T cells from patients with rheumatoid arthritis, *PFKFB3* is lost, leading to decreased T-cell glucose consumption and impaired autophagy, which in turn lead to an inability to mount a normal immune response and an increase in T-cell apoptosis (37). Further studies are thus warranted to investigate the role of *PFKFB3* in LADA and to determine whether this signal is truly a distinguishing feature between adult and childhood-onset autoimmune diabetes.

Although the lead genome-wide significant loci are shared with those for type 1 diabetes risk, they clearly have a diminished impact in LADA. To further investigate the differences between LADA and type 1 diabetes at the HLA region, we performed a comparative haplotype analysis that showed a decreased frequency of type 1 diabetes–associated risk haplotypes in LADA. This could be partly explained by the established age gradient in HLA frequencies seen in patients with type 1 diabetes (38); however, HLA risk genotype frequencies have also been shown to differ between patients with LADA and patients with type 1 diabetes with age at onset older than 35 years (14,39). Future in-depth studies of the differences in HLA risk haplotypes between type 1 diabetes and LADA, taking age and ethnicity into account, are therefore also warranted.

In terms of type 2 diabetes–associated loci, our results differ from previous candidate studies. For instance, our previously reported *HNF1A* (12) locus was not observed in this setting. Furthermore, although previous studies showed an association for the leading type 2 diabetes risk locus at *TCF7L2* with LADA (11,16), our data show relatively limited support of this finding (Supplementary Table 10) (LADA vs. population control subjects, rs7903146-T: OR [95% CI] = 1.107 [1.024–1.20]; $P = 0.011$), which may be due to the limited power of our study to detect type 2 diabetes signals (Supplementary Table 2). To understand the evidence supporting the previous association, we examined the allele frequencies of the lead variant in each contributing cohort. This revealed

that the difference in risk allele frequency between case and control subjects was cohort specific, with only one case-control set (Action LADA case vs. BMDCS control subjects) not supporting this association, principally due to the higher frequency of the risk allele in the control set (Supplementary Table 13). One possibility is that inclusion or exclusion of patients with type 2 diabetes from control cohorts would affect the frequency of the risk allele; however, sensitivity analysis with control sets that either excluded or included patients with diabetes in Swedish and Danish samples showed the persistence of an association (Supplementary Table 13), although not at the genome-wide significance level. Interestingly, a recent study found that the type 2 diabetes risk allele at the key *TCF7L2* locus was associated with case subjects with type 1 diabetes who were older than 12 years at onset and positive for only a single autoimmune antibody (40). That study provides further evidence for a role for type 2 diabetes genetic risk in later-onset autoimmune diabetes and resonates with the genome-wide observations we report in this study in adults.

The precise diagnostic criteria used to distinguish LADA from adult-onset type 1 and type 2 diabetes remain under debate. These differences in opinion have hindered the collection of well-phenotyped, clearly defined LADA cohorts for genetic studies and are reflected in the cohorts we included in this study (e.g., in terms of heterogeneous age inclusion thresholds and differences in autoantibody testing). In this study, we strove to be inclusive to maximize our sample size and statistical power, but we acknowledge that stringent, deeply phenotyped cohorts are needed to truly address where adult autoimmune diabetes is placed on the diabetes spectrum. Another debate surrounds the idea that LADA cohorts may simply be collections of poorly phenotyped case subjects with adult-onset type 1 and type 2 diabetes and refutes the idea that LADA is a unique disease entity. However, GADA assays have a specificity of 95–98%, so by implication, some case subjects with type 2 diabetes with low-level GADA can be incorrectly classified as case subjects with LADA; these would, however, represent only a very small fraction of case subjects because the predictive specificity of GADA would have been increased by our

cohort enrichment as with any biomarker assay. Conversely, the small percentage of case subjects with LADA who do not have GADA positivity but have other islet autoantibodies and are misclassified as having type 2 diabetes could affect the estimate of genetic correlation between LADA and type 2 diabetes to a small degree. Future studies should focus on defining the heterogeneity and misdiagnosis rates among patients with LADA.

Despite these limitations, using the definition of LADA presented in this study, we identified factors that potentially distinguish this form of adult autoimmune diabetes from childhood-onset type 1 diabetes as well as type 2 diabetes: 1) a novel signal at the *PFKFB3* locus and 2) attenuation of type 1-associated HLA risk haplotypes. Overall, we find the presence of both a type 1 diabetes-like autoimmune genetic component and a type 2 diabetes-like metabolic genetic component consistent with the phenotypic features of both main diabetes types, suggesting that LADA as defined in this study is a hybrid of these two major diseases. Our findings promote the hypothesis that the polygenic component that contributes susceptibility to type 2 diabetes can act as a modifier to type 1 diabetes risk, possibly as a “second hit” in individuals who have moderate underlying autoimmune susceptibility that is insufficient to trigger childhood type 1 diabetes but greater than that of the general population and sufficient to lead to clinical diabetes in adulthood. Taken together, future studies should examine the role of BMI, which is lower in type 1 diabetes and higher among patients with type 2 diabetes, in adult autoimmune diabetes, as well as further defining the role of factors that potentially distinguish adult autoimmune diabetes from type 1 and type 2 diabetes.

Conclusion

In this first GWAS of LADA, we show that the leading genome-wide significant signals point toward LADA as being a late-onset form of type 1 diabetes, albeit with a genetically attenuated potency of key type 1 diabetes-associated HLA haplotypes, but also with a type 2 diabetes-like genetic component. Further in-depth studies are necessary to address how LADA and insulin dependence develop and to study the impact of heterogeneity among case subjects with LADA, as

well as a need for functional studies to investigate how the glycolytic regulator *PFKFB3* is situated at the intersection of autoimmune and metabolic diabetes. Furthermore, our LADA data set should act as a resource to help mitigate the unaccounted presence of autoimmune diabetes in patients masquerading as type 2 diabetes, with implications for both GWAS and clinical management.

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